

EFFECTS OF AN INTENSIVE LIFESTYLE INTERVENTION PROGRAM ON PORTAL HYPERTENSION IN PATIENTS WITH CIRRHOSIS AND OBESITY: THE SPORTDIET STUDY

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ABSTRACT

Obesity increases the risk of clinical decompensation in cirrhosis, possibly by increasing portal pressure. Whether weight reduction can be safely achieved through lifestyle changes (diet and exercise) in overweight/obese patients with cirrhosis, and if weight loss reduces portal pressure in this setting is unknown. This prospective, multicentric, uncontrolled pilot study enrolled patients with compensated cirrhosis, portal hypertension (hepatic venous pressure gradient, HVPG ≥ 6 mmHg) and body mass index (BMI) ≥ 26 Kg/m² in an intensive 16-week lifestyle intervention program (personalized hypocaloric normoproteic diet and 60 min/wk of supervised physical activity). We measured HVPG, body weight and composition, adipokines, health-related quality-of-life and safety data prior and after the intervention. Changes in HVPG and body weight were pre-defined as clinically relevant if $\geq 10\%$ and $\geq 5\%$, respectively. Safety and body weight were re-assessed after 6 months. 60 patients were included and 50 completed the study (56 \pm 8 y/o; 62% male; NASH etiology 24%; BMI 33.3 \pm 3.2 Kg/m²; Child A 92%; HVPG ≥ 10 mmHg 72%). Lifestyle intervention significantly decreased body weight (average -5.0 \pm 4.0 Kg; $p < 0.0001$), by $\geq 5\%$ in 52% and $\geq 10\%$ in 16%. HVPG also significantly decreased (from 13.9 \pm 5.6 mmHg to 12.3 \pm 5.2 mmHg, $p < 0.0001$), by $\geq 10\%$ in 42% and $\geq 20\%$ in 24%. A $\geq 10\%$ body weight loss was associated with a greater decrease in HVPG (-23.7 \pm 19.9% vs. -8.2 \pm 16.6%, $p = 0.024$). No episodes of clinical decompensation occurred. Weight loss achieved at 16-wks was maintained at 6-month; Child and MELD scores did not change.

Conclusions. 16-weeks of diet and moderate exercise were safe and reduced body weight and portal pressure in overweight/obese patients with cirrhosis and portal hypertension.

INTRODUCTION

Obesity and obesity-associated conditions are growing health problems worldwide. In recent prospective studies over 70% of patients with compensated cirrhosis were either overweight or obese (1, 2), similarly to the general population (1, 2). Besides being a cause of chronic liver disease *per se*, obesity worsens the prognosis of patients with compensated cirrhosis induced by other causes (1, 2), and the risk of first clinical decompensation of cirrhosis is approximately three times higher in obese patients as compared to normal weight ones (1, 2). The mechanisms explaining this clinical association have not been clarified yet, but appears to involve a progressive increase in portal pressure (1). Since portal hypertension constitutes the pathophysiological basis of most complications of cirrhosis (3), therapeutic measures for patients with cirrhosis should be aimed at reducing portal pressure. In particular, non-pharmacological strategies to improve portal hypertension would be highly desirable(4).

Weight loss $\geq 5\text{-}7\%$ obtained by combining hypocaloric diet and exercise is an effective treatment for obesity-related chronic liver disease (non-alcoholic fatty liver disease, NAFLD)(5, 6); furthermore, such a relatively modest weight loss is sufficient to determine clinically relevant improvements in other clinical scenarios, such as a reduction of cardiovascular risk in overweight and obese patients with type 2 diabetes (7). Mechanisms involved include improvement in insulin-resistance and reduction in proinflammatory, profibrogenic and pro-angiogenic adipokines/cytokines. Some of these cytokines, such as Leptin, have been suggested to modulate portal hypertension in cirrhosis; we have recently shown that leptin receptor blockade decreased hepatic

vascular resistance and portal pressure in experimental cirrhosis (8), which supports the view that excessive leptin release might exacerbate portal hypertension in obese cirrhotics.

However, the safety of weight loss in patients with cirrhosis has not been fully established yet, and there is concern regarding possible deleterious effects on liver function mediated by worsening of sarcopenia (9).

Given the above mentioned evidence, we hypothesized that an intensive life-style intervention consisting in a personalized and monitorized hypocaloric diet and supervised moderate physical exercise would result in improvement in portal hypertension in patients with compensated cirrhosis and overweight or obesity; this effect would be mediated by a decrease in intrahepatic vascular resistance. Furthermore, intensive life-style intervention would be associated with a modest body weight loss without adverse events on liver function.

The present study was aimed at testing these hypotheses.

METHODS

Selection of Patients

From September 2011 through November 2013 we conducted a prospective, multicentric, pilot study in six University Hospitals in Spain. The study was registered in ClinicalTrials.gov (registration number: NCT 01409356).

All the following inclusion criteria had to be fulfilled:

- Liver cirrhosis of any aetiology diagnosed by histology or by clear clinical, laboratory and imaging criteria
- compensated stage or single episode of variceal bleeding > 6 months before inclusion, provided this was not associated with ascites or any other complication
- Child-Pugh class A or B \leq 8 points
- HVPG \geq 6 mmHg
- BMI \geq 26 Kg/m², stable in the last 6 months (BW changes < 3 Kg were considered acceptable)
- Age 18-75 years

Safety considerations: since exercise can be associated with acute increases in portal pressure (10), all included patients with gastroesophageal varices had to be on proper, stable primary or secondary prophylaxis of variceal bleeding. An electrocardiogram was obtained in all patients on the screening visit.

Any of the following was considered exclusion criteria: previous or ongoing ascites; previous or ongoing jaundice, severe bacterial infections leading to hospitalisation, porto-systemic encephalopathy, hepatocellular carcinoma. Active alcohol consumption (minimum abstinence: 6 months) untreated large gastroesophageal varices; complete portal vein thrombosis; Child-Pugh Score >8; transjugular intrahepatic porto-systemic

shunt; previous liver transplantation; ischemic heart disease or electrocardiographic signs of ischemic heart disease; severe orthopaedic problems limiting the possibility to exercise.

Ethical aspects

This study, coordinated by the Hospital Clinic in Barcelona, was approved by the Ethics Committee of each participating center. The nature of the study was explained to the patients, and a written informed consent was obtained in each case, according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000).

Endpoints

The main endpoints of this study were the changes in body weight and in HVPG after 16 weeks of intensive lifestyle intervention.

Secondary endpoints included safety (liver-related events and changes in liver function tests), and changes in body composition, oxygen consumption, adipokines and health-related quality of life after 16 weeks of lifestyle intervention.

Variables of the study

All the variables object of the study were assessed on inclusion and after completing 16 weeks of lifestyle intervention.

A diagram illustrating the study design is provided in Supplementary Figure 1.

Clinical and laboratory data. Clinical history data included etiology of liver disease, presence of esophageal varices and ongoing treatment with non selective beta-blockers, presence of diabetes, arterial hypertension, and dyslipemia, and concomitant medication; hemodynamic assessment included simple data on systemic hemodynamics: heart rate, systolic, diastolic and mean pressure (evaluated by standard sphygmomanometry after 10 minute rest on the day of the visit); laboratory data

included standard tests (hemoglobin, leucocytes count, platelet count, AST/ALT, GGT, Alk. Phosphatase, Creatinine, Bilirubin, Albumin, Glucose, Sodium, Potassium).

Plasma and serum samples were obtained and stored for adipokines, adipocytokines and cytokines testing. Interleukin 6 (IL-6); Interleukin 8 (IL-8), Interleukin 1beta (IL-1b), Insulin, Leptin, Hepatocyte Growth Factor (HGF), Tumor Necrosis Factor alpha (TNF-a), Nerve Growth Factor (NGF) and Monocyte Chemoattractant Protein 1 (MCP-1) were simultaneously measured on these samples by Immunoessay (see Suppl. Methods).

Hepatic Venous Pressure Gradient was measured according to the international recommendations as previously described (see Suppl. Methods)(11).

Indocyanine green clearance, extraction index and hepatic blood flow were measured in a subgroup of 23 patients, included in two of the participating centers, as previously described (12) (see Suppl. Methods).

Nutritional status assessment was made analyzing the dietary habits of the patients; anthropometric measurement included weight, height, waist circumference and fat mass (bioelectrical impedance analysis, BIA) (see Suppl. Methods).

Physical activity assessment. Patients were asked specific questions regarding whether they used to perform exercise, and in case they did regarding its kind, intensity and duration; specific questions regarded sedentarism (number of hours spent watching TV or computer). A subgroup of patients (n=24, all included in Barcelona) underwent assessment of maximal oxygen uptake (VO₂max) by a standard Bruce's test based on progressive effort on a treadmill at baseline and 16-wks. An indirect estimation of maximal oxygen uptake was used in patients in whom Bruce's test was not available (13).

Quality of life. A liver-disease specific validated questionnaire was used (CLDQ, Spanish edition)(14). Patients filled-in autonomously the questionnaire, which contains questions regarding 6 domains of quality of life (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function, worry). Score for every question is an entire number between 0 (the worst possible) and 7 (the best possible).

Lifestyle intervention

An intensive lifestyle intervention was initiated within one week of the baseline HVPG measurement. It consisted in 16-week of caloric restriction (diet) and exercise, which are shortly detailed below (see also the Supplementary Methods).

Diet: consisted in a tailored reduction in caloric intake of 500-1000 kCal per day.

Protein intake was maintained between 20 and 25% of the total intake in order to reduce the risk of muscle mass loss, but within 0.8 g/Kg of ideal BW (calculated for a BMI=25 Kg/m²) per day. Diet was balanced in order to be not ketogenic, and as varied as possible. Carbohydrates accounted for 45-50% of total kCal, fat content was maintained < 35% of total kCal and 20 g of alimentary fiber was also recommended. No alcohol was allowed.

Physical activity: On the same week of nutritional intervention initiation, patients began exercising. Exercise was supervised by two professional trainers (PTs), one for patients included in Barcelona and one for patients included in Madrid. One weekly 60 minutes session of moderate exercise in small groups (1-5 patients) was conducted. Every session consisted in 10 minutes of warm up, 40 minutes of aerobic and strength exercising routine, and 10 minutes of cooling down. Exercise routine was chosen by the trainers avoiding abdominal workouts/pump, and tailored to a perceived exertion of 4-5/10 (Visual Analogue Scale) or 10-12 Borg Rating of Perceived Exertion Scale.

In addition, all patients were instructed to increase their daily standard physical activity.

The total amount of physical activity over the 16 weeks of the study was estimated by calculating the metabolic equivalents of tasks (METs) (15).

Clinical follow-up: once a month the patient was seen by one of the participating hepatologists in order to assess changes in the clinical status, record any relevant event and changes of medication occurring during the study. An extra visit was scheduled 6 months after the completion of the 16-weeks lifestyle intervention in order to evaluate medium-term safety and body weight.

Sample size

According to previous data in patients with cirrhosis, changes in HVPG were pre-defined as clinically relevant if $\geq 10\%$ (16). As for changes in body weight, we pre-defined it as clinically relevant if $\geq 5\%$ (17). Based on data obtained in non-cirrhotic patients with obesity (18), we assumed that a maximum of 50% of the included patients would be able to decrease body weight to this extent. Since no data regarding changes in HVPG associated with body weight changes were available previous to this study, we hypothesized that 30% of patients showing a clinically relevant weight loss would also show a clinically relevant reduction in HVPG. With these assumptions, accepting an alpha error of 0.05, a beta error of 0.20 and 10% subject loss during the study, using the Fischer exact test, the inclusion of 60 patients was required to demonstrate the existence of a clinical association between weight loss and reduction in HVPG.

Statistical analysis

A minimum attendance of 30% to the lifestyle intervention visits was required to enter the final analysis. Quantitative variables are expressed as mean and standard deviation

(SD), whereas qualitative variables as absolute and relative frequencies. Comparisons between groups were made by the Student's T test, Mann-Whitney test or Kruskal-Wallis test, as appropriate for quantitative variables, and by Chi square, Fisher's test for the qualitative variables. Within each group continuous variables were compared using the Student's t test for paired data. Correlations between variables were computed using the Pearson's or Spearman Rho coefficient, when appropriate. Statistical analysis was performed with SPSS 22.0 package (SPSS Inc., Chicago, IL USA). The α value was set at 0.05. All p-values are two-sided.

All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

60 patients were considered eligible and signed the informed consent; 3 of them were not put on the lifestyle intervention program due to HVPG < 6 mmHg (inclusion error).

Among the 57 patients who initiated the program, 7 either did not complete it (withdrawal of consent, n= 2; suspicion/identification of hepatocellular carcinoma on ultrasound during the study, n=2) or did not fulfil the minimal pre-defined attendance criteria (n=3).

50 patients successfully completed the study and were included in the final analysis.

Their main characteristics are summarised in Table 1. The attendance to physical activity sessions and nutritional therapy session was high, respectively 88% and 84%.

Adherence to calory intake was $75 \pm 14\%$.

As for non-supervised physical activity, 39/50 patients correctly completed the physical activity log over 16 weeks. Among them, 16 engaged in weekly or biweekly exercising in addition to walking, while for 23 walking was the only additional exercise. Overall, the median cumulative walking/exercising time was 123.7 hours (range: 43 hours- 241 hours) and the calculated METs were 400.5 (150.5-1032.9) (Supplementary Table 1).

Effect of intensive lifestyle intervention on body weight and body composition

The intensive lifestyle intervention induced a significant decrease in body weight (Figure 1; Supp. Figure 2), which was on average -5.0 ± 4.0 Kg ($p < 0.0001$); the decrease was $\geq 5\%$ (clinically relevant) in 52% of the included population; in 16% weight loss was $\geq 10\%$. Changes in body weight were similar in the different etiologies of cirrhosis: viral: -6.7 ± 3.8 Kg ($p < 0.0001$ vs. baseline); alcohol: -5.5 ± 4.8 Kg ($p < 0.0001$ vs. baseline); NASH: -4.1 ± 4.0 Kg ($p = 0.002$ vs. baseline) (Kruskall-Wallis test among etiologies: $p = 0.587$).

Weight loss was associated with a significant decrease in fat mass, while lean mass did not change (Table 2). Body weight loss was maintained after 6 months of follow-up (86.2 ± 13.7 Kg at 16-wk vs. 85.6 ± 13.7 Kg after 6 months, $p=0.257$). At this time-point about half of the population referred to have continued on diet (57%) and exercise (53%) after completing the 16 weeks of intensive LS intervention.

Effect of lifestyle intervention on HVPG

HVPG significantly decreased after 16-wk of lifestyle intervention (Figure 2, Supp. Figure 3), from 13.9 ± 5.6 mmHg to 12.3 ± 5.2 mmHg ($p<0.0001$). A clinically relevant decrease in HVPG (namely $\geq 10\%$ vs. baseline) was observed in 42% of the included population; in 24% HVPG decreased $\geq 20\%$. Furthermore, 4 patients with HVPG ≥ 10 mmHg on inclusion showed a HVPG value < 10 mmHg after 16-wk LS intervention.

In the subset of 23 patients that had measurements of hepatic blood flow, similarly to what observed in the whole included population, there was a significant decrease in HVPG (15.7 ± 5.4 mmHg at baseline vs. 13.7 ± 5.1 mmHg at 16 weeks; $p=0.015$), which was not associated with any change in hepatic blood flow (898 ± 520 ml/min at baseline vs. 782 ± 382 at 16 weeks; $p=0.565$), but which was associated with a significant improvement in indocyanine green extraction (from 0.54 ± 0.17 to 0.59 ± 0.19 ; $p=0.048$).

These findings suggest that a decrease in intrahepatic vascular resistance contributed to the decrease in portal pressure.

Relation of changes in body weight and in HVPG

Figure 3 summarises the observed changes in HVPG according to the observed reduction in body weight. Overall, there was no statistical correlation between changes in body weight and HVPG ($R=0.010$, $p=0.944$). However, patients achieving a greater reduction in body weight, namely $\geq 10\%$, exhibited a greater decrease in HVPG (

-23.7±19.9% vs. -8.2±16.6% decrease in HVPG in those reducing body weight less than 10%; $p=0.024$). Interestingly, the 4 patients showing a reduction in HVPG below the threshold of 10 mmHg had decreased their body weight $\geq 10\%$.

Relation of changes in HVPG and cumulative physical activity during the study period

This point was addressed in the 39 patients who provided data regarding the amount of non-supervised physical activity they carried out over the study period. A more intense physical activity, namely above the median of the study group (METs: 400.5 over 16 weeks; Supplementary Table 1), was associated with a more marked decrease in HVPG: -16.7±17.3% in patients above the METs median vs. -5.1±19.9 in patients exercising below the METs median; $p=0.054$. As shown in Table 3, the beneficial effect of exercise resulted statistically significant only in patients showing non-clinically relevant changes in body weight. These results suggest a possible independent effect of body weight reduction and physical activity on portal pressure.

Relation of changes in HVPG and body weight with other variables

The presence of diabetes and a higher fat mass on BIA were associated, respectively, to lack of clinically relevant reduction of body weight and to lack of clinically relevant reduction of HVPG after the intensive lifestyle intervention (Table 1). Patients with diabetes showed a lower change in HVPG as compared to non-diabetic patients (% change in HVPG: -5.2±16.7% vs. -14.7±17.9%; $p=0.06$).

HVPG and body weight decreased similarly in patients on non-selective beta-blockers (NSBBs) vs. those not taking NSBBs (Table 1). The adherence to diet of patients on NSBB was similar to that of patients not on NSBB (median: 73.1% vs. 81.2%, NS), and the median hours spent walking/exercising over 16 weeks was similar as well (122±43 hours vs. 122±53 hours, NS). No differences were seen in the improvement of indirectly

assessed maximal aerobic capacity of patients on NSBB (from baseline 28.5 ± 5.3 to 31.7 ± 5.6 ml/Kg/min after LS intervention, $p < 0.0001$) and not on NSBB (from baseline 28.3 ± 5.6 to 30.9 ± 6.1 ml/Kg/min after LS intervention, $p < 0.0001$).

Having experienced or not an episode of variceal bleeding, cirrhosis etiology, severity of portal hypertension, presence/absence of arterial hypertension or dyslipidemia did not influence these results. Among the baseline variables, on a multivariate analysis including fat mass and diabetes, only fat mass remained negatively and independently associated with HVPG response to LS intervention (B coefficient - 0.129; $p = 0.030$).

Changes in insulin, adipokines/adipocytokines and cytokines

Most of the tested plasma levels of cytokines, namely IL-1, IL-6, IL-8, HGF, NGF, TNF α and MCP1, did not change after the LS intervention. Serum insulin and leptin significantly decreased after LS intervention (Table 2), being their decrease greater in patients showing a clinically relevant weight loss. Insulin decreased by $33 \pm 30\%$ in patients with weight loss $\geq 5\%$ vs. $5 \pm 55\%$ in those with weight loss $< 5\%$ ($p = 0.043$). As a consequence, HOMA decreased from 9.5 ± 9.4 to 6.1 ± 4.8 , $p = 0.015$, and QUICKI increased from 0.29 ± 0.03 to 0.31 ± 0.04 , $p = 0.008$. Similarly, leptin decreased by $42 \pm 25\%$ in patients with weight loss $\geq 5\%$ vs. $4 \pm 43\%$ in those with weight loss $< 5\%$ ($p = 0.001$). Anyhow, changes in cytokines were similar in patients who showed a clinically relevant HVPG decrease vs. those who did not.

Changes in quality of life

Quality of life significantly improved after LS intervention: global score: 5.5 ± 1.0 vs. 5.2 ± 1.0 at baseline ($p < 0.001$). A significant improvement was noticed in all the domains of the questionnaire (Supplementary Figure 4); the improvement was

particularly evident in patients achieving a clinically relevant weight loss: global score:

5.7±1.0 vs. 5.3±1.1 at baseline, $p<0.0001$ (all domains).

Safety

None of the patients experienced episodes of clinical decompensation during the 16 weeks of lifestyle intervention. Child and MELD scores did not change after 16 weeks of diet and exercise (Table 2). Adverse event during the 16 weeks of LS intervention were few: 1 case of mild pretibial edema, spontaneously disappeared; 1 reported worsening of asthma requiring increase in bronchodilators dosage, and 1 episode of arthritis due to gout. On the other hand, 4 patients (one on insulin alone, two on insulin + metformin and 1 on metformin alone) required reduction in their medication for diabetes and 1 patient stopped spironolactone for disappearance of lower limbs edema.

DISCUSSION

Obesity incidence is increasing dramatically worldwide, and is nowadays common in patients with compensated cirrhosis (1, 2). It has been previously shown that obesity has a negative impact on the natural history of cirrhosis (1, 2), but very little is known about how these two chronic conditions interact. More specifically it is unknown whether standard measures correcting obesity, that have a positive effect on many of its metabolic and cardiovascular consequences (19), can also improve the course of cirrhosis through improving portal hypertension.

The major finding of this study is that a short (4 months) intensive lifestyle changes intervention, consisting in tailored diet and supervised exercise of moderate intensity, is able to reduce portal pressure in patients with cirrhosis and overweight or obesity.

Importantly, the lifestyle intervention proved safe, since no episode of clinical decompensation was observed during the study, and liver function tests remained unchanged.

Over 40% of the included patients showed a clinically relevant benefit on portal pressure, defined as a decrease in HVPG $\geq 10\%$ of baseline. Such a decrease has been shown to be of clinical relevance preventing ascites in patients on primary prophylaxis with non-selective beta-blockers (16), and was associated with a decreased formation of varices in compensated portal hypertensive patients without esophageal varices (20).

Furthermore, the decrease in HVPG was $\geq 20\%$ of baseline in a quarter of the included patients, a magnitude of reduction that has been associated with a decrease in the incidence of all complications of portal hypertension and with improved survival when achieved on chronic non-selective beta-blockers (21-23). The amount of weight loss showed no linear relationship with the degree of HVPG reduction, suggesting that the

mechanisms leading to the reduction in portal pressure are likely complex and may differ between patients; however, patients achieving a greater weight loss, namely >10% of body weight, also showed a significantly greater HVPG reduction, including achieving more frequently a >10% decrease in HVPG and reaching a final HVPG below of 10 mmHg (the risk threshold for developing complications from portal hypertension). These results might justify recommending a weight loss of at least 10% in this population, in order to maximise the likelihood of a clinically relevant benefit on portal pressure. As for the possibility of predicting which patients would benefit more from a lifestyle intervention, we observed no significant influence of etiology, severity of portal hypertension, presence/absence of previous episodes of variceal bleeding and presence/absence of arterial hypertension or dyslipidemia; however, the presence of diabetes was associated with a lower reduction in body weight and in HVPG, suggesting that in this population other approaches should be attempted. Interestingly, a higher fat mass on inclusion was associated with lack of clinically relevant reduction of HVPG after the intensive lifestyle intervention. This finding is intriguing and points to the possibility that adipose tissue mass rather than BMI is important in the modulation of portal hypertension. However, it has been previously showed that the study of body composition by BIA has limitations in patients with cirrhosis, mostly due to erratic measurements in case of water retention (24). Since our population excluded patients with previous or ongoing ascites, it is unlikely that water retention could have prevented a correct evaluation of the effect of BMI and its changes on portal pressure.

Patients on NSBBs showed an attendance to exercising and an improvement in maximal aerobic capacity similar to that of patients not on NSBBs, consistent to results obtained in non-cirrhotic subjects showing that NSBBs do not prevent a training-induced

amelioration of VO₂ (25). Notably, the positive effect of diet and exercise on HVPG was observed also in patients already on NSBBs, confirming that this non-pharmacological approach leads to an added benefit in cirrhosis. This finding further suggests that the mechanism of the reduction in HVPG achieved through the life-style intervention differs from that of non-selective beta-blockers (splanchnic vasoconstriction)(3). Indeed, it is worth noting that in the subgroup of our patients that had measurements of hepatic blood flow before and after life-style intervention, no significant change was observed; a decrease in HVPG in the context of such a lack of changes in liver blood flow is another strong argument indicating a decrease in hepatic vascular resistance. Also, as discussed below, the positive effect of weight loss on insulin resistance and leptin release probably resulted in a fall in hepatic resistance and in this way mediated the decrease in HVPG observed after weight loss (8, 26).

It should be underlined that our study included only patients without ascites and without any previous episodes of ascites; this was done to ensure homogeneity of the population and to avoid the potential detrimental effect of weight loss in patients that are almost invariably sarcopenic (9). On the other hand, it is important to observe that in the present study weight loss was not associated to a reduction in lean mass (mostly muscle mass), likely due to the protective effect of exercise on muscle mass (27) and of the adequate protein intake provided by the tailored diet. Despite further studies in this field, our results suggest that in order to avoid worsening or onset of sarcopenia in obese patients with cirrhosis in whom body weight reduction is attempted, exercise needs to be combined to calorie restriction, and diet macronutrients should include an appropriate amount of proteins.

Due to the pragmatic design of the study, that included both tailored diet and exercise, we cannot answer the question whether the observed effect on HVPG is due to only one of them, and regarding the specific role of each of the two components of lifestyle intervention. According to data obtained in patients with NAFLD/NASH, the combination of diet and exercise is more effective in achieving a significant weight loss, but exercise per se seems sufficient to reduce the amount of visceral fat (27). Anyhow, in a subgroup of our study population, we observed that a higher amount of hours spent exercising during the study period was associated with a more pronounced decrease in HVPG, and that exercise may possibly explain HVPG reduction in some patients not achieving a clinically relevant weight loss (Figure 3; Table 3). Even if preliminary, our data suggests that physical activity per se might modulate portal pressure in cirrhosis. The present study has some limitations; some of them are inherent to any exploratory pilot study, without randomised allocation to intervention or to a control group. Nonetheless, the likelihood that the HVPG spontaneously decreased in the included patients is very low, as proved by several studies addressing hemodynamic changes in compensated cirrhosis not undergoing etiological treatment. Of note, this study was conducted before the new generation of direct antiviral agents for hepatitis C was available in Spain. We cannot provide firm data on the molecular mechanisms leading to portal pressure decrease; however, our findings lead us to propose that the decrease in insulin and in particular of leptin, could mediate changes in portal pressure occurring over a relatively short period due to their ability to modulate the intrahepatic vascular resistance (Figure 4). Insulin (and insulin-resistance expressed either as HOMA or QUICKI indexes) and leptin overall decreased after the lifestyle intervention, and markedly decreased in patients who lost $\geq 10\%$ of body weight. It is worth noting that

we have recently reported that leptin-receptor blockade decreases hepatic vascular resistance and portal pressure in experimental cirrhosis(8), and that the correction of insulin-resistance by metformin has a similar effects (26). This reinforces the view that reduced leptin levels and improved insulin-sensitivity through life-style intervention may be part of the mechanisms involved in the decrease in HVPG observed in our patients. On the other hand, it should be noted that the decrease in insulin and leptin levels did not differ between patients showing a $\geq 10\%$ decrease in the HVPG or not. Therefore, our findings suggest that although metabolic changes are likely to be involved, they cannot fully explain our observations. We cannot provide data on the expression of these cytokines on liver tissue, since repeat liver biopsies were not done due to ethical and economical reasons; therefore the mechanism linking changes in body weight to portal hypertension remain an open field for future studies. Pure NASH-related cirrhosis (without other concomitant etiologic factors) was present in one fourth of patients included in this study. This small sample size did not allow us providing data regarding a possible (and likely) more marked effect of LS intervention on portal pressure in this etiology.

Another limitation of the study is that we cannot assess whether the benefit of the life-style intervention would be maintained, improved or lost over a long-term follow-up, as the observation period was limited to the 4-month of life-style intervention plus 6 months of follow-up. It is possible that the beneficial effect hereby reported fades over time in case of new increase in body weight and/or sedentary life; however, it is equally likely that reiterated intervention might maintain the beneficial effect. It is worth noting in this regard that the success of the program in terms of achieving a body weight loss

of >5% exceeded all expectations (mostly derived from the obesity/diabetes field). It is likely that the excellent attendance and adherence to the program was partly due to the its intensive nature (with 10 nutritionist visits over 4 months and weekly sessions with a personal trainer) but we believe largely reflect the marked benefits on the perceived quality of life reported by the patients, which is likely to explain why the weight loss persisted unmodified during the 6 months of follow-up. Future studies should address whether new electronic tools such as electronic Fitness trackers (bracelets, watches, or clip-ons) might help in maintaining a good adherence to lifestyle changes lifelong.

In conclusion, an intensive 16-week programme of tailored diet and moderate exercise can be safely recommended to obtain weight loss and HVPG decrease in overweight/obese patients with compensated cirrhosis and portal hypertension, and can be considered a useful non-pharmacological intervention in this population.

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Figure Legends.

Figure 1. Effects of the intensive lifestyle intervention on body weight in the study population. As shown, a significant decrease was achieved, and 52% of patients showed a body weight decrease $\geq 5\%$ (a priori defined as “clinically relevant”).

Figure 2. Effects of the intensive lifestyle intervention on HVPg in the study population. As shown, a significant decrease was achieved, and 42% of patients showed a HVPg decrease $\geq 10\%$ (a priori defined as “clinically relevant”).

Figure 3. Percentage of change in HVPg according to the percentage of change in body weight. As shown, patients with weight loss over 10% showed a more pronounced decrease in HVPg, while patients in whom body weight did not change or increased did not show any change in HVPg. * indicates $p < 0.05$ vs. baseline values.

Figure 4. Graphical summary of the hypothetical effect of lifestyle intervention on HVPg in patients with cirrhosis and obesity.

References

1. Berzigotti A, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Morillas R, Escorsell A, et al. Obesity is an independent risk factor for clinical decompensation in patients with cirrhosis. *Hepatology* 2011;54:555-561.
2. Everhart JE, Lok AS, Kim HY, Morgan TR, Lindsay KL, Chung RT, Bonkovsky HL, et al. Weight-related effects on disease progression in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Gastroenterology* 2009;137:549-557.
3. Bosch J, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol* 2015;62:S121-130.
4. de Franchis R, Baveno VIF. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015.
5. Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. *J Hepatol* 2015;62:S65-S75.
6. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, et al. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015;149:367-378 e365; quiz e314-365.
7. Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, Hill JO, et al. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care* 2011;34:1481-1486.

8. Delgado MG, Gracia-Sancho J, Marrone G, Rodriguez-Vilarrupla A, Deulofeu R, Abraldes JG, Bosch J, et al. Leptin receptor blockade reduces intrahepatic vascular resistance and portal pressure in an experimental model of rat liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2013;305:G496-502.
9. Salo J, Guevara M, Fernandez-Esparrach G, Bataller R, Gines A, Jimenez W, Gines P, et al. Impairment of renal function during moderate physical exercise in cirrhotic patients with ascites: relationship with the activity of neurohormonal systems. *Hepatology* 1997;25:1338-1342.
10. Garcia-Pagan JC, Santos C, Barbera JA, Luca A, Roca J, Rodriguez-Roisin R, Bosch J, et al. Physical exercise increases portal pressure in patients with cirrhosis and portal hypertension. *Gastroenterology* 1996;111:1300-1306.
11. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009;6:573-582.
12. Navasa M, Garcia-Pagan JC, Bosch J, Rodes J. [Prognostic value of hepatic clearance of indocyanine green in patients with liver cirrhosis and hemorrhage of esophageal varices]. *Med Clin (Barc)* 1992;98:290-294.
13. Nes BM, Vatten LJ, Nauman J, Janszky I, Wisloff U. A simple nonexercise model of cardiorespiratory fitness predicts long-term mortality. *Med Sci Sports Exerc* 2014;46:1159-1165.
14. Ferrer M, Cordoba J, Garin O, Olive G, Flavia M, Vargas V, Esteban R, et al. Validity of the Spanish version of the Chronic Liver Disease Questionnaire (CLDQ) as a standard outcome for quality of life assessment. *Liver Transpl* 2006;12:95-104.

15. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, Greer JL, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011;43:1575-1581.
16. Hernandez-Gea V, Aracil C, Colomo A, Garupera I, Poca M, Torras X, Minana J, et al. Development of ascites in compensated cirrhosis with severe portal hypertension treated with beta-blockers. *Am J Gastroenterol* 2012;107:418-427.
17. Blackburn G. Effect of degree of weight loss on health benefits. *Obes Res* 1995;3 Suppl 2:211s-216s.
18. Williams RL, Wood LG, Collins CE, Callister R. Effectiveness of weight loss interventions--is there a difference between men and women: a systematic review. *Obes Rev* 2015;16:171-186.
19. Bruix J, Gores G, Mazzaferro V. Authors' response to the letter: Liver resection for patients with hepatocellular carcinoma and macrovascular invasion, multiple tumours or portal hypertension by Zhong et al. *Gut* 2014.
20. Groszmann RJ, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, et al. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005;353:2254-2261.
21. D'Amico G, Garcia-Pagan JC, Luca A, Bosch J. Hepatic vein pressure gradient reduction and prevention of variceal bleeding in cirrhosis: a systematic review. *Gastroenterology* 2006;131:1611-1624.
22. Abraldes JG, Tarantino I, Turnes J, Garcia-Pagan JC, Rodes J, Bosch J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology* 2003;37:902-908.

23. Feu F, Garcia-Pagan JC, Bosch J, Luca A, Teres J, Escorsell A, Rodes J. Relation between portal pressure response to pharmacotherapy and risk of recurrent variceal haemorrhage in patients with cirrhosis. *Lancet* 1995;346:1056-1059.
24. Moctezuma-Velazquez C, Garcia-Juarez I, Soto-Solis R, Hernandez-Cortes J, Torre A. Nutritional assessment and treatment of patients with liver cirrhosis. *Nutrition* 2013;29:1279-1285.
25. Juhlin-Dannfelt A. beta-Adrenoceptor blockade and exercise: effects on endurance and physical training. *Acta Med Scand Suppl* 1983;672:49-54.
26. Tripathi DM, Erice E, Lafoz E, Garcia-Caldero H, Sarin SK, Bosch J, Gracia-Sancho J, et al. Metformin reduces hepatic resistance and portal pressure in cirrhotic rats. *Am J Physiol Gastrointest Liver Physiol* 2015;309:G301-309.
27. Berzigotti A, Saran U, Dufour JF. Physical activity and liver diseases. *Hepatology* 2016;63:1026-1040.

Table 1. Baseline characteristics of included patients.

Variable	Overall (n=50)	Showing BW decrease \geq 5% after LS intervention (n=26)	Showing BW decrease <5% after LS intervention (n=24)	P	Showing HVP decrease $\geq 10\%$ after LS intervention (n=21)	Showing HVP decrease <10% after LS intervention (n=29)	P
Age, yrs	56 \pm 8	56 \pm 8	56 \pm 8	0.841	57 \pm 7	55 \pm 9	0.318
Males, n (%)	31 (62%)	15 (58%)	16 (67%)	0.570	16 (76%)	15 (52%)	0.139
Etiology of cirrhosis, viral/Alcohol/NASH/Autoimmune, n	18/19/12/1	11/9/5/1	7/10/7/0	0.674	5/8/7/1	13/11/5/0	0.334
Gastroesophageal Varices present, n (%)	33 (66%)	16 (62%)	17 (71%)	0.559	12 (57%)	21 (72%)	0.366

Previous variceal bleeding, n (%)	15 (30%)	7 (27%)	8 (33%)	0.760	6 (29%)	9 (31%)	1.000
On chronic non-selective beta-blockers, %	30 (60%)	16 (67%)	14 (58%)	1.000	11 (52%)	19 (66%)	0.393
HVPG, mmHg	13.9 ± 5.6	13.3 ± 5.0	14.5 ± 6.2	0.457	14.9 ± 6.2	13.1 ± 5.1	0.252
% with HVPG≥10 mmHg	72	69	75	0.757	71	72	1.000
Child class A, %	92	92	92	1.000	90	93	1.000
Platelet count, n*10 ⁹ /mmc	106±48	103±41	110±55	0.615	96±31	113±57	0.223
Glucose, g/dl	124±48	112±28	136±61	0.084	120±65	126±32	0.669
AST, U/L	48±41	49±38	47±45	0.837	50±39	47±43	0.758
ALT, U/L	44±37	44±37	43±37	0.928	47±40	41±34	0.594
Bilirubin, mg/dl	1.1±0.6	1.1±0.6	1.1±0.5	0.985	1.1±0.6	1.1±0.5	0.867
Albumin, g/L	36±5	37±5	36±5	0.492	36±5	37±5	0.548
International Normalised Ratio	1.19±0.20	1.22±0.22	1.18±0.18	0.564	1.24±0.22	1.17±0.18	0.223

Creatinine, mg/dl	0.73±0.14	0.74±0.12	0.72±0.15	0.712	0.73±0.15	0.73±0.12	0.955
MELD score	9±3	9±3	9±2	0.727	9±3	9±2	0.438
MAP, mmHg	92±13	91±14	94±12	0.412	90±13	94±13	0.423
Heart Rate, beats per minute	66±13	66±14	66±11	0.792	64±12	67±13	0.369
Body weight, Kg (range)	91.5 ± 13.9 (64.4-118.4)	91.4 ± 15.8	91.6 ± 11.7	0.957	93.2 ± 14.1	90.2 ± 13.7	0.456
BMI, Kg/m2 (range)	33.3 ± 3.2 (27.6-39.9)	33.2 ± 3.3	33.3 ± 3.1	0.970	33.0 ± 3.2	33.4 ± 3.2	0.618
BMI 26-29.9 (Overweight), n (%)	7 (14%)						
BMI 30-34.9 (Class I obesity), n (%)	26 (52%)						
BMI 35-.39.9 (Class II obesity), n (%)	17 (34%)						
Waist circumference, cm	114 ±11	113 ±12	114 ±10	0.810	114 ±10	114 ±12	0.947
Men	119±9						

Women	106±1						
% Fat Mass	37.8 ± 7.6	38.7 ± 6.6	36.7 ± 8.4	0.389	34.5 ± 8.0	40.1 ± 6.3	0.013
Men	35.6 ± 7.7						
Women	41.4 ± 6.0						
Completely sedentary lifestyle, n (%)	32 (64%)	17 (65%)	15 (62%)	0.772	12 (57%)	19 (66%)	0.570
Daily intake of sugar drinks, n (%)	20 (40%)	8 (31%)	12 (50%)	0.248	9 (43%)	11 (38%)	0.776
Estimated daily caloric intake, kCal	2100 ± 450	2080 ± 460	2120 ± 440	0.752	2140 ± 430	2080 ± 470	0.650
Men	2265 ±400						
Women	1800 ± 380						
Diabetes mellitus, n (%)	21 (42%)	6 (23%)	15 (63%)	0.009	7 (33%)	14 (48%)	0.387
Treatment for diabetes, n							
Diet only/Metformin							
only/Sulphonylurea only/Insulin	1/12/2/1/5	1/2/1/0/2	0/10/1/1/3	0.342	1/4/0/1/1	0/8/2/0/4	0.249
only/Metformin+Insulin							

% of each treatment (% refers to diabetic patients)	5/57/10/5/23	17/33/17/0/33	0/67/7/7/20		14/57/0/14/14	0/57/14/0/28	
History of arterial hypertension, n (%)	23 (46)	11 (42%)	12 (50%)	0.777	9 (43%)	14 (48%)	0.778
Treatment for art. hypertension , n	18	8	10	0.640	8	10	0.610
History of Dyslipidemia, n (%)	8 (16)	4 (15%)	4 (17%)	1.000	3 (14%)	5 (17%)	1.000
Treatment for dyslipemia, n	5	1	4	0.142	2	3	1.000
	(Simvastatin in all)						

Table 2. Results of the variables object of the study before and after 16 weeks of intensive lifestyle intervention.

	Baseline	After 16- wk LS interv.	p
HVPG, mmHg	13.9±5.6	12.3±5.2	<0.0001
Heart rate, bpm	66±13	63±13	0.031
Hepatic Blood Flow, ml/min*	898±520	782±382	0.565
Indocyanine green extraction index, %	54±17	59±19	0.048
Systolic arterial pressure, mmHg	127±18	125±18	0.283
Diastolic arterial pressure, mmHg	71±11	70±9	0.637
Mean arterial pressure, mmHg	90±12	88±11	0.393
Body weight, Kg	91.5±13.9	86.5±13.6	<0.0001
Waist Circumference, cm	114 ±11	107±12	<0.0001
Fat Mass, Kg	35.1±9.1	30.2±8.3	<0.0001
Lean Mass, Kg	56.7±11.2	55.6±12.7	0.340
Total Cholesterol (mg/dl)	161±34	149±34	<0.0001

Tryglicerides (mg/dl)	105±88	86±33	0.147
Glucose, mg/dl	124±48	118±48	0.406
HOMA	9.5±9.4	6.1±4.8	0.015
QUICKI	0.29±0.03	0.31±0.04	0.008
Child score	5.5±0.7	5.6±0.9	0.229
MELD score	9.0±2.6	9.4±2.8	0.152
Albumin, g/L	37±5	36±5	0.387
Bilirubin, mg/dl	1.13±0.55	1.17±0.71	0.482
INR	1.20±0.20	1.20±0.21	0.711
Creatinine, mg/dl	0.73±0.13	0.73±0.19	0.889
Platelet count, 10⁹/L	106±48	105±56	0.793
Oxygen uptake-VO₂, mL/kg/min**	21.3±7.4	25.7±9.3	<0.0001
Plasma insulin, pg/ml	912±726	670±506	0.002
Plasma leptin, ng/ml	29.7±18.3	20.3±12.7	<0.0001

* available in 23 of the included patients. ** available in 24 of the included patients.

Table 3. Percentage HVPG Change after 16 weeks of LS intervention according to the amount of BW reduction and to the amount of exercise performed (METs). Data refer to the 39 patients in whom physical activity could be quantified. Data are expressed both as median (interquartile range). Non-parametric tests were used to obtain the p value.

HVPG Decrease			
	Exercised above median	Exercised below median	P
BW Decrease >10%	- 16.3 (N/A) (n=3)	- 24.3 (47.7) (n=4)	0.582
BW Decrease 5-10%	-8.7 (15.5) (n=8)	0 (13.2) (n=9)	0.196
BW Decrease <5%	-15.0 (21.3) (n=8)	0 (25.5) (n=7)	0.014

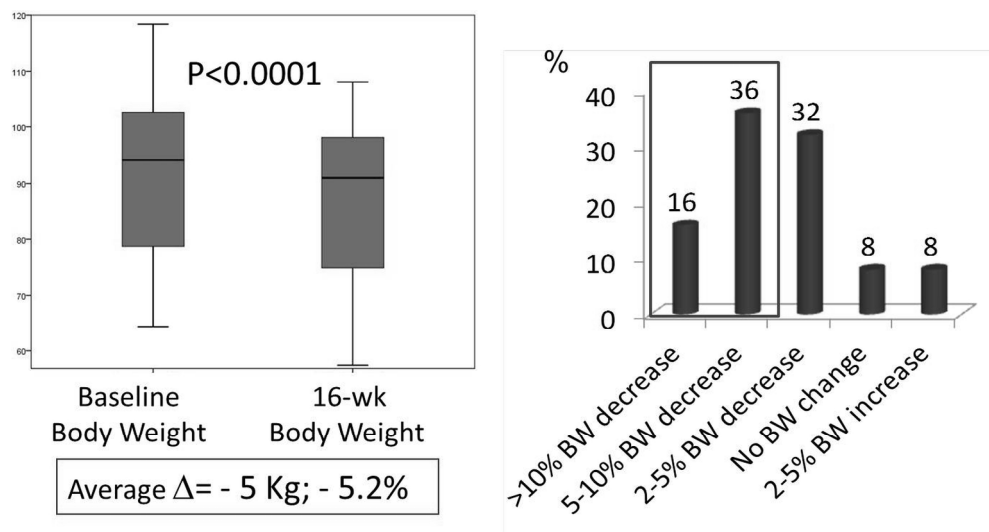


Figure 1

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Accepted

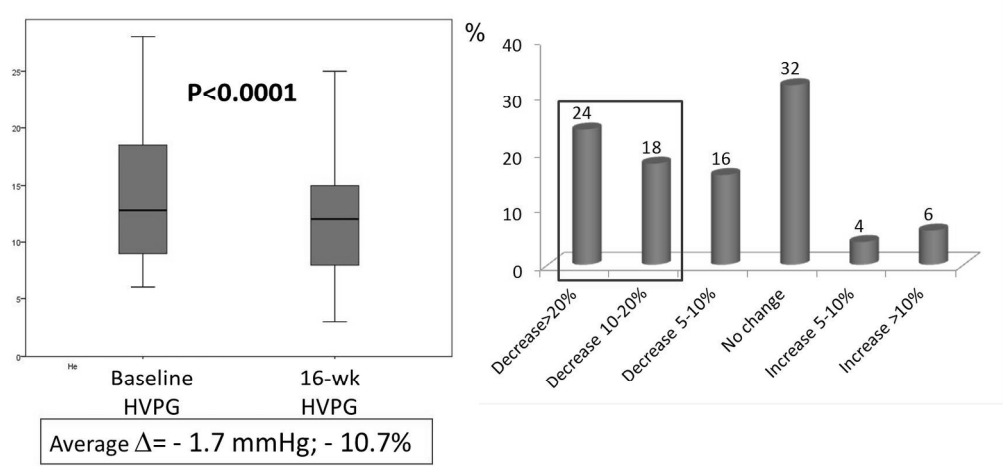


Figure 2

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Accepted

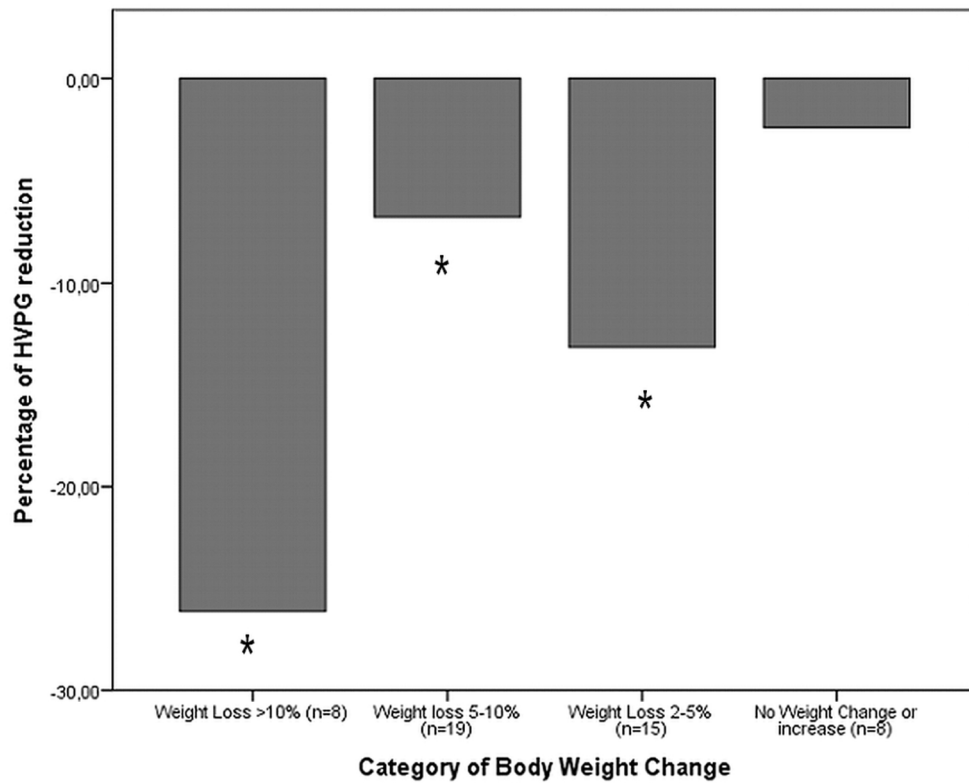


Figure 3

150x120mm (300 x 300 DPI)

Accepted A

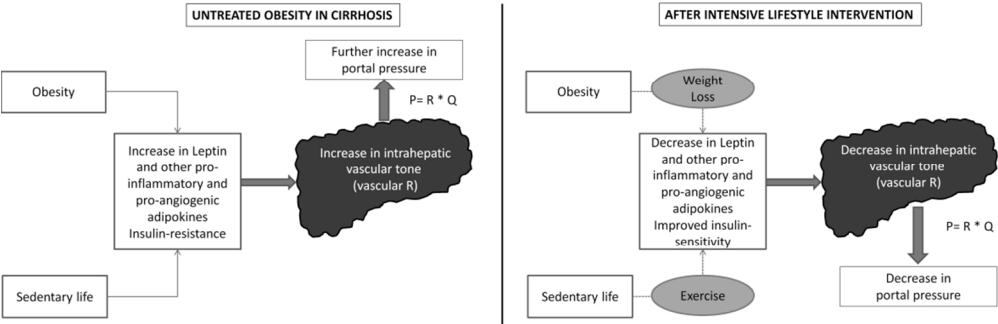


Figure 4

99x32mm (300 x 300 DPI)

Supplementary Methods

Measurement of Adipokines, adipocytokine and cytokines: – A commercial multiplexed bead-based immunoassay Milliplex MAP Human Adipokine Magnetic Bead Panel 2 (HADK2MAG-61K, Merck Millipore, Darmstadt, Germany) was used to simultaneously measure the following 9 biomarkers in serum on a Luminex 100 Bioanalyzer (Luminex Corp., Austin, TX): Interleukin 6 (IL-6); Interleukin 8 (IL-8), Interleukin 1beta (IL-1b), Insulin, Leptin, Hepatocyte Growth Factor (HGF), Tumor Necrosis Factor alpha (TNF-a), Nerve Growth Factor (NGF) and Monocyte Chemoattractant Protein 1 (MCP-1). The readouts were analyzed with the standard version of the Milliplex Analyst software (Merck Millipore). A five-parameter logistic regression model was used to create standards curves (pg/mL) and to calculate the concentration of each sample.

Human adiponectin levels were determined in 25 µl of serum using the specific ELISA kit EZHADP-61k (Merck Millipore).

Hepatic Venous Pressure Gradient. In the morning, after fasting overnight, patients were transferred to the hepatic hemodynamic laboratory. Under local anesthesia, a 8F venous catheter introducer was placed in the right internal jugular vein using the Seldinger technique. Under fluoroscopic control a 7F balloon-tipped catheter (Edwards Lifesciences, Irvine, CA, USA) was advanced into the right hepatic vein to measure wedged and free hepatic venous pressures using pre-calibrated electro-mechanical transducer and polygraph (Mac-Lab®, GE Healthcare, Freiburg, Germany). The wedged position was obtained by inflating the balloon and confirming the occlusion of the hepatic vein by injecting a small amount of contrast medium. HVPG was calculated

as the difference between wedged and free hepatic venous pressures (1). All measurements were performed in triplicate and permanent tracings were recorded (1). The free hepatic vein pressure was measured at 1-3 cm of the ostium of the hepatic vein into the inferior vena cava (1).

Hepatic blood flow.

A subgroup of 23 patients (those included in two of the participating centers: one center in Barcelona and one center in Madrid) underwent measurement of hepatic blood flow through indocyanine green clearance method. Preceded by a priming dose of 5 mg, a solution of indocyanine green (Pulsion Medical Systems, Munich, Germany) was infused intravenously at a constant rate of 0.2 mg/min. After an equilibration period of at least 40 minutes to achieve a steady-state, 4 separate sets of simultaneous samples of peripheral and hepatic venous blood were obtained for the measurement of hepatic blood flow according to the Fick's method as previously described. To avoid interferences from differences in plasma turbidity, the Nielsen's correction was used at the moment of reading ICG concentration in the samples by spectrophotometry (SP-830, Turner Biosystems, Sunnyvale, CA, USA).

Briefly, ICG clearance was calculated as ICG constant infusion velocity/mean concentration of ICG in the peripheral venous blood. ICG extraction index was calculated as: (concentration of ICG in the peripheral venous blood – concentration of ICG in the hepatic venous blood)/concentration of ICG in peripheral venous blood. Hepatic plasma flow was estimated as ICG clearance/ICG extraction index. Finally, hepatic blood flow was estimated as: hepatic plasma flow /(1- hematocrit) (2).

Nutritional status assessment. Patients were asked to fill-in a 3-days log (2 working days and 1 week-end day) regarding their dietary habits, which was assessed by a

experienced nutritionist in order to calculate the baseline food intake of the patient (total Kcal, grams and % of carbohydrates, lipids and proteins; water intake; fiber intake in grams). The following anthropometric measures were also recorded: waist circumference, body weight. Bioelectrical impedance analysis method was used to assess lean/fat mass (Tanita body composition analyzer, Model BC-148 MA, Tanita Corp., Tokyo, Japan).

Physical activity assessment. Patients were asked specific questions regarding whether they used to perform exercise, and in case they did regarding its kind, intensity and duration; specific questions regarded sedentarism (number of hours spent watching TV or computer). Evaluation of maximal oxygen consumption (VO₂) was performed by the Bruce test in 24 patients, all included in Barcelona.

Quality of life

A liver-disease specific validated questionnaire was used (CLDQ, Spanish edition)(3). Patients filled-in autonomously the questionnaire, which contains questions regarding 6 domains of quality of life (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function, worry). Score for every question is an entire number between 0 (the worst possible) and 7 (the best possible).

Lifestyle intervention

An intensive lifestyle intervention was initiated within one week of the baseline HVPG measurement. It consisted in 16-week of caloric restriction (diet) and exercise, which are detailed below.

Diet: consisted in a tailored reduction in caloric intake of 500-1000 kCal per day (according to the European Food Information Council this kind of diet allows a weight loss of 0,7 - 1,4 Kg per week, sufficient to achieve a clinically relevant weight loss).

Tailoring consisted taking into account the alimentary preferences and preferred intake hours of each patient, in order to maximize adherence. Protein intake was maintained between 20 and 25% of the total intake in order to reduce the risk of muscle mass loss, but within 0.8 g/Kg of ideal BW (calculated for a BMI=25 Kg/m²)/day. Diet was balanced in order to be not ketogenic, and as varied as possible. Carbohydrates accounted for 45-50% of total kCal, fat content was maintained < 35% of total kCal, and 20 g of alimentary fiber was also recommended. No alcohol was allowed. The nutritionist provided to patients examples of food schemes (usually 3 major meals and 2 snacks), also indicating food that could be exchanged with other with similar nutritional content, and asked patients to monitor weekly their BW, at home or in a pharmacy, in order to increase their awareness. After the first visit patients were followed-up by the nutritionist once a week for the first month (intensification period), and every two weeks thereafter. At each visit patients were given a new three-day nutritional log to be filled-in in order to increase their awareness regarding food intake. This was checked by the nutritionist during the next follow-up visit; data were entered a software (Dietsource 3.0 ® Nestle Healthcare Nutrition S.A., Vevey, Switzerland) that allows quantifying caloric intake. Assessment of adherence to diet was performed by a subjective tool (visual analogue scale) and by an objective quantification as follows:

adherence in percentage = $[100 - (\text{real calculated caloric intake} - \text{recommended intake} / \text{recommended intake}) * 100]$.

At the end of each visit the nutritionist gave verbal positive reinforcement about dieting and exercising.

Physical activity: On the same week of nutritional intervention initiation, patients began exercising. Exercise was supervised by two professional trainers (PTs), one for patients

included in Barcelona and one for patients included in Madrid. One weekly 60 minutes session of moderate exercise in small groups (1-5 patients) was conducted. Every session consisted in 10 minutes of warm up, 40 minutes of aerobic and strength exercising routine, and 10 minutes of cooling down. Exercise routine was chosen by the trainers avoiding abdominal workouts/pump, and tailored to a perceived exertion of 4-5/10 (Visual Analogue Scale) or 10-12 Borg Rating of Perceived Exertion Scale.

A subgroup of patients (n=24, all included in Barcelona) underwent assessment of maximal oxygen uptake (VO₂max) by a standard Bruce's test based on progressive effort on a treadmill at baseline and 16-wks.

In addition, all patients were instructed to increase their daily standard physical activity, e.g. by walking at least 30 minutes, and progressively as long as possible, and/or by swimming or biking. The PTs suggestion was to avoid excessive efforts, defined as > 6/10 of that perceived subjectively. Patients were given an exercise log to fill-in every day to increase their awareness and adherence. The PTs reviewed each patient's log at every weekly session and gave verbal positive reinforcement of the importance of maintaining and improving daily physical activity.

References

1. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009;6:573-582.
2. Navasa M, Garcia-Pagan JC, Bosch J, Rodes J. [Prognostic value of hepatic clearance of indocyanine green in patients with liver cirrhosis and hemorrhage of esophageal varices]. *Med Clin (Barc)* 1992;98:290-294.
3. Ferrer M, Cordoba J, Garin O, Olive G, Flavia M, Vargas V, Esteban R, et al. Validity of the Spanish version of the Chronic Liver Disease Questionnaire (CLDQ) as a standard outcome for quality of life assessment. *Liver Transpl* 2006;12:95-104.

Supplementary Table 1. Unsupervised physical activity per week and over the entire LS intervention in the study subgroup with available data (n=39). Data is shown as median (minimum-maximum).

Activity	Median Hours/week (min-max)	Overall Hours spent for the activity over 16 weeks	Metabolic Equivalents of Tasks
Walking (all patients, n=39)	5.1 (1.9-14.3)	97.9 (43.0-241.0)	NA
Subgroup performing other physical activities (n=16) Cycling: n=10, swimming n= 2, jogging n=1 and mixed aerobic/anaerobic activity n=3	2 (0.5-8)	30 (4-151)	NA
Total walking or exercising over 16 weeks (n=39)	NA	123.7 (43-241)	400.5 (150.5-1032.9)

Appendix

Ciberehd SportDiet Collaborative Group: **Hospital Clinic, Barcelona:** Hepatologists: Annalisa Berzigotti; Jaume Bosch; Juan Carlos García-Pagán; Juan G. Abraldes; Joan Caballería; Jordi Colmenero; Alejandro Forner; Enric Reverter; Susana Seijo; Nutritionists: Violeta Moize, Alba Andreu; Nurses at Hepatic Hemodynamics Laboratory: Angels Baringo, Laura Rocabert, Rosa Saez, Lara Orts; Biochemists: Joan Claria, Esther Titos. **Hospital Santa Creu i Sant Pau, Barcelona:** Hepatologists: Candid Villanueva; Alba Ardevol; Isabel Graupera; Nutritionists: Pilar Ulldemolins; Amelia Romero. **Hospital Universitari Vall d'Hebron, Barcelona:** Hepatologists: Joan Genescá; Salvador Agustín; Nutritionist: Guillermo Cardenas; Research nurse: Laura Millán. Personal Trainer for all the patients included in the three Centers in Barcelona: Marco Mangone. **Hospital Univ. Ramón y Cajal, Madrid:** Hepatologists: Agustín Albillos; Francisco Mesonero. **Hospital Puerta de Hierro, Madrid:** Hepatologists: Jose Luis Calleja; Elba Llop. **Hospital General Universitario Gregorio Marañón, Madrid:** Hepatologists: Rafael Bañares; Cristina Ripoll; Javier García-Lledó. Nutritionist and Personal Trainer for the patients included in the three Centers in Madrid: Belén Rodríguez

Supplementary Figure 1. Diagram illustrating the study design.

Supplementary Figure 2. Individual changes in Body weight obtained the intensive LS

intervention. The red line indicates the average value of the studied population: from 91.5 ± 13.9 Kg to 86.5 ± 13.6 Kg ($p < 0.0001$).

Supplementary Figure 3. Individual changes in HVPG obtained the intensive LS

intervention. The red line indicates the average value of the studied population: from 13.9 ± 5.6 mmHg to 12.3 ± 5.2 mmHg ($p < 0.0001$).

Supplementary Figure 4. Effects of the intensive LS intervention on the different domains of CLDQ.

INTENSIVE LIFE STYLE INTERVENTION:**Hepatology**

Diet – hypocaloric (-500/1000 kcal/day; usually 1200-1400 kcal/day), 25% proteins

+

Physical exercise – supervised moderate exercise
60 min/week + advice to avoid sedentary life
(activity diary)
Nutritionists visits every 2 weeks

- 1- Inclusion/exclusion criteria
- 2- Informed consent
- 3- Electrocardiogram ok

**BASELINE:**

- 1) Nutritionists: BW; height
waist circumference;
bioelectric
impedentiometry
(fat/lean mass)
- 2) HVPg, arterial pressure, HR
- 3) Blood samples
- 4) CLDQ

**Follow-up visits
(physician)**

POST-INTERVENTION:

- 1) Nutritionists: BW; waist
circumference; bioelectric
impedentiometry
(fat/lean mass)
- 2) HVPg, arterial pressure, HR
- 3) Blood samples
- 4) CLDQ

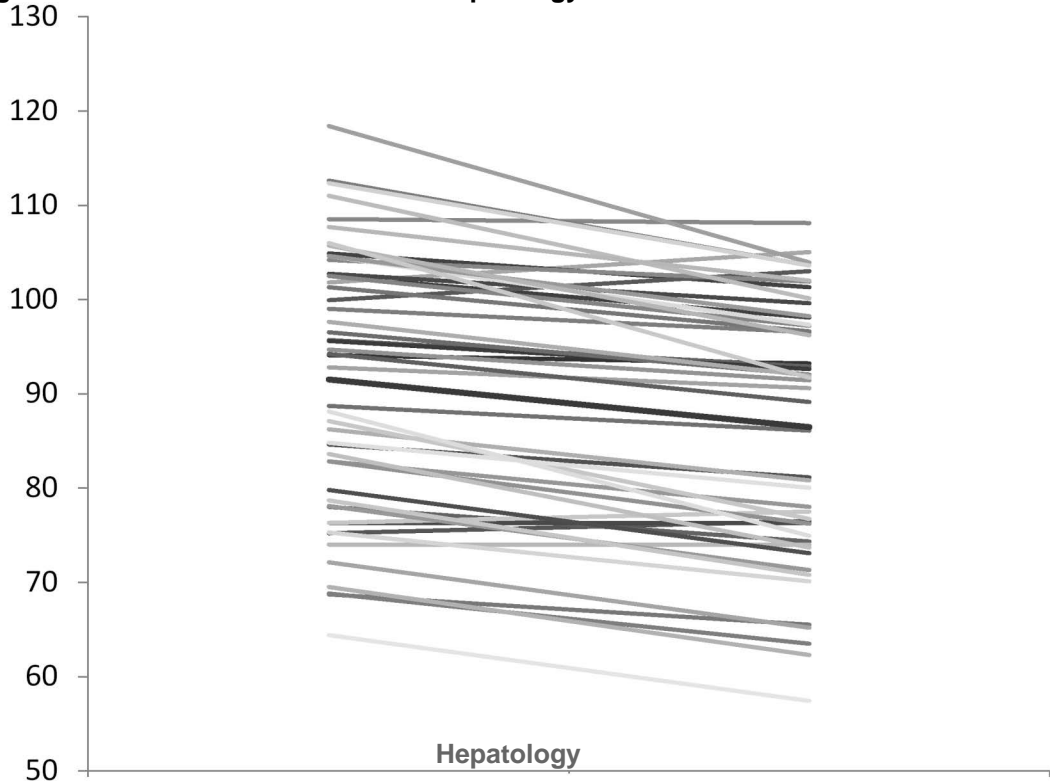
END OF STUDY

- 1) Clinical events
- 2) BW
- 3) Maintenance of diet and
exercise

Hepatology

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Hepatology



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